REMARKS

Claims 1-4, 6, and 8-14 are pending.

Applicants acknowledge the Examiner's rejection of claims 1-4, 6, 8-11, 13-14, and 16, under 35 U.S.C. § 112, first paragraph, as allegedly lacking sufficient enablement. Applicants have provided rebuttal arguments and claim amendments to obviate this rejection.

Applicants acknowledge the Examiner's rejection of claims 1-4, 6, 8-11, and 13-14, under 35 U.S.C. § 103(a) as allegedly obviated by Dennis et al. (U.S. application 2003/0044388) (hereinafter "Dennis") in view of Heiskanen et al. (*Cancer Research* 60:799, 2000) (hereinafter "Heiskanen"). Applicants respectfully traverse this rejection because neither Dennis nor Heiskanen teaches use of organ-specific methylation patterns as presently claimed.

No new matter has been added.

Rejections under 35 U.S.C. § 112, first paragraph

The Examiner rejected claims 1-4, 6, 8-11, 13-14, and 16 under 35 U.S.C. § 112, first paragraph, as allegedly lacking sufficient enablement.

Specifically, discussing the *WANDS* factors (1-8, already of record) and reciting the various elements of Applicants' claims, the Examiner states that "while the art does enable one of skill in the art to analyze cytosine methylation in free floating DNA, neither the art nor the specification enables one of skill in the art to determine the presence of a cancer based upon an increased amount of organ specific free floating DNA."

Breadth of claims. The Examiner reiterates Applicants' claim elements, and concludes that "the claims are drawn to detection of the presence or an increase amount of free floating DNA originating from a particular organ and correlating the detection with the presence of any cancer characterized by an increased amount of organ-specific free floating DNA as compared to a normal control value" (emphasis added).

Nature of the Invention. The Examiner states that the claims broadly encompass "determining any DNA methylation pattern for any organ and detection of the presence of any cancer" (emphasis added). The Examiner states that "the claims broadly encompass determining the presence of any cancer condition that originates from any organ by detection of the presence of any type of methylation pattern" (emphasis added). The Examiner further states that "the invention is in a class of invention which the CAFC has characterized as 'the unpredictable arts such as chemistry and biology' (citing Mycogen Plant Sci., Inc. v. Monsanto Co)."

In response, Applicants have amended claim 1 to recite:

> "obtaining a bodily fluid sample from a test human having a cancer in a particular organ characterized by an increased amount of corresponding organ-specific free floating DNA relative to that of a normal control value;
>
> measuring an amount or presence of free floating DNA that originates from the[[a]] particular organ in the sample comprising analysing for a DNA methylation pattern that is characteristic for the particular organ; and
>
> comparing the measured amount or presence of free floating DNA that originates from the particular organ of the test human with that of a normal control value, and determining the presence of a cancer in the particular organ characterized by an increased amount of organ-specific free floating DNA based on an increased measured amount of corresponding organ-specific free floating DNA."

Conforming amendments have also been made to claims 2, 6, 10, 11, and 13.

Contrary to the Examiner's statement that that "the claims broadly encompass determining the presence of any cancer condition that originates from any organ by detection of the presence of any type of methylation pattern," the current amendments serve to clarify that the determined cancer is limited to a cancer in a particular organ characterized by an increased amount of corresponding organ-specific free floating DNA relative to that of a normal control value, and that the organ-specific methylation pattern that is measured corresponds to that of the particular cancerous organ.

Teachings of specification and state of the art. The Examiner states that "the specification asserts a means to predict which organ has developed a medical condition, by employing means of distinguishing between DNA originating from different healthy or different organs of the human body (page 19, last paragraph). The specification asserts characteristic methylation patterns of certain genes can be positively correlated with specific organs (page 19, last paragraph)," and further "does not provide a *predictive association* of the detection of any cancer by the detection of methylation patterns" (emphasis added). The Examiner states that

"the specification indicates that to determine if methylation patterns are associated with cancer a comparison study must be done, however, the claims as broadly written merely comprise the detection of methylation patterns."

The Examiner concludes (citing post-filing art; *Cottrell*, hereinafter "Cottrell") that, based on the specification and teachings in the art, it is unpredictable to correlate the methylation pattern of any free floating DNA to ANY cancer condition by detecting methylation patterns (or merely DNA) because the art teaches "lack of predictability with regard to methylation pattern studies and correlation to any cancer condition." Finally, the Examiner states that the specification teaches that the correlation of disease and free-floating DNA "must have an association step to compare to a normal individual and potentially a validation step."

In response, Applicants point out that organ specific methylation patterns are used and not cancer-specific methylation patterns. Moreover, as stated above, claim 1 has been amended to clarify that that the determined cancer is limited to a cancer in a particular organ characterized by an increased amount of corresponding organ-specific free floating DNA relative to that of a normal control value, and that the organ-specific methylation pattern that is measured corresponds to that of the particular cancerous organ.

With respect to the Examiner's comments on Cotttrell, Applicants agree that methylation-based studies must have adequate requirements for consistency and performance, and defined clinical questions, sample sets, and methodologies coupled with current methylation technology. Indeed, Applicants maintain their contention that the teachings of the instant specification in combination with the skill in the art provide these benchmark requirements. Cottrell merely emphasizes the importance of precisely the approaches disclosed by the present Applicants. Applicants respectfully point out that the unpredictability associated with technical/methodological issues for detection of methylation differences were sufficiently

overcome as of time of filing of the present application. The present Applicants are recognized in the art for highly industrial, sophisticated array-based processes that allow for the simultaneous analysis of thousands of CpG sequences or multiple indications in an efficient, high-throughput manner. Moreover, Applicants have amended the claims as described above to limit the claims to detection of cancer of particular organs and that are accompanied by an increased level of the particular organ-specific DNA in the blood or body fluid.

The predictability or unpredictability of the art and degree of experimentation. The Examiner states that art teaches that there is unpredictability in associating circulating DNA (free floating) with cancer (citing Bremnes, Jung, Raykan, and Sidransky). The Examiner states (citing the Abstract of Bremnes) that large clinical trials are needed to validate and standardize tests for DNA alteration in plasma for individuals at risk for, or having lung cancer. The Examiner states (citing Yates) that methylation is not only caused by neoplasms, but that methylation can be detected in normal tissue (e.g., from aged individuals), and that detection of methylation does not, therefore, necessarily indicate neoplastic tissue. The Examiner states that "Lui et al. (Clin Chem Lab Med 2002 Vol. 40 p. 962-968) (hereinafter "Lui") teaches that circulating DNA is present in increased amounts in transplant patients (page 963, last two paragraphs, and page 964, first paragraph) and in trauma patients (page 964, second paragraph). Therefore the presence of cancer is not the only source of circulating DNA in the body." The Examiner states that "Eckhardt et al. [hereinafter "Eckhardt"] teaches methylation patterns are influenced by a number of endogenous and exogenous parameters (page 1381, first column, last paragraph)."

Significantly, however, Applicants respectfully point out that the altered ***methylation levels*** discussed in Bremnes were <u>not ***methylation patterns*** being correlated with specific organs</u>

as presently claimed and, therefore, that the Examiner's use of Bremnes is unsupportable. The level of methylation per se, or whether the entire amount of circulating DNA correlates to cancer or not is irrelevant to the method as claimed, because it is the amount of *organ-specific* circulating DNA, which is the analyte of interest, and which is correlated to the presence of a diseased organ. With respect to Eckhardt, Applicants point out that variation in methylation patterns with age or reflecting heterogenous cell types within a tissue does not contradict the fact that tissue specific methylation patterns exist, and moreover a showing of a stronger correlation of between age and methylation in e.g., lung and colon does not against the presently claimed utility of organ specific methylation patterns—nowhere does Eckhardt teach against the presently claimed utility of organ specific methylation patterns. With respect to the teachings of Raykan, Applicants again point out that the altered ***methylation levels*** discussed in Raykan were not ***methylation patterns*** being correlated with specific organs as presently claimed, and therefore, that the Examiner's use of Raykan is unsupportable. Finally, with respect to Dennis, Applicants respectfully point out that the methylation pattern detected is not a tissue specific methylation pattern, but rather is a cancer-specific methylation pattern of the cancer in the transplanted tissue, such the Examiner's use of Dennis is misplaced and unsupportable.

Amount of direction or guidance provided by the specification. The Examiner states that "the specification does not provide any specific guidance as to how to correlate detection of any cancer by the detection of free floating DNA. The specification discloses that a correlation to cancer must include an association step to compare methylation patterns to individuals and a validation study to confirm detection of cancer," and that "the art teaches detection of cancer with methylation patterns in free floating DNA is unpredictable and that these associations need to be confirmed by multiple large sampling sizes to determine a clear association."

The current claim amendments serve to clarify that the determined cancer is limited to a cancer in a particular organ characterized by an increased amount of corresponding organ-specific free floating DNA relative to that of a normal control value, and that the organ-specific methylation pattern that is measured corresponds to that of the particular cancerous organ.

The specification also does not indicate that a correlation *must* include a *validation study* to confirm detection of disease.

Working Examples. The Examiner states that the specification provides no examples to correlate detection of disease by detection of free floating DNA in any individual, because no "p-value" is provided (citing Example 1 and Figure 7), or statistical significant association, and that the specification does not have an example of determining in ANY sample a correlation of methylation pattern with detection of ANY cancer condition. The Examiner states that "Further, the guidance in the specification only indicates that an increased level of organ specific free floating DNA is indicative of an organ based cancer, but not a specific cancer. Further, it is well known that some cancer have effects on multiple organs. For example, Paredes-Zaglul et al. (Clinical Cancer Research Vol. 4 April 1998 p. 879) (hereinafter "Paredes-Zaglul") teaches that colon cancer spreads to the liver (page 879, second column, first paragraph). Therefore it would be unclear if an elevated free floating DNA amount in the liver would be indicative of a liver based cancer or rather a colon based cancer."

The current claim amendments serve to clarify that the determined cancer is limited to a cancer in a particular organ characterized by an increased amount of corresponding organ-specific free floating DNA relative to that of a normal control value, and that the organ-specific methylation pattern that is measured corresponds to that of the particular cancerous organ. The claim language, therefore, requires only that that cancer (of whatever type) be in a particular

organ characterized by an increased amount of corresponding organ-specific free floating DNA relative to that of a normal control value.

Quantity of Experimentation. The Examiner states that the quantity of experimentation needed is extremely large, because the artisan would need to the association of detection of disease with measurement of free floating DNA, determine if the association was species based, and that this would require significant effort to practice the invention as presently claimed.

Applicants disagree as discussed in more detail below.

Level of skill in the art. The Examiner states that the level of skill in the art is deemed to be high.

Conclusion by Examiner. The Examiner concludes that despite the level of skill in the art being high, given the specification guidance and working example, it would require *undue* experimentation to practice the invention as claimed.

Applicants respectfully traverse the Examiner's enablement rejection in view of Applicants' present claim amendments, the applicable law, and Applicants rebuttal arguments above and below.

Applicants' maintained traversal:

Applicants' present amendments serve to clarify that the determined cancer is limited to a cancer in a particular organ characterized by an increased amount of corresponding organ-specific free floating DNA relative to that of a normal control value, and that the organ-specific methylation pattern that is measured corresponds to that of the particular cancerous organ. Based on the amendments, Applicants respectfully traverse the Examiner's rejection, because the

proper inquiry with respect to scope of enablement under 35 U.S.C. §112, first paragraph, is whether it would require *undue* experimentation to make and use the subject matter as claimed, and, as discussed below in detail, such is not the case.

Relevant Law:

Applicants maintain that to satisfy the enablement requirement of 35 U.S.C. §112, first paragraph, the specification must teach one of skill in the art to make and use the invention without *undue* experimentation. Atlas Powder Co. v. E.I. DuPont de Nemours, 750 F.2d 1569, 224 USPQ 409 (Fed. Cir., 1984). This requirement can be satisfied by providing sufficient disclosure, either through illustrative examples or terminology, to teach one of skill in the art how to make and how to use the claimed subject matter without undue experimentation. This clause does not require "a specific example of everything within the scope of a broad claim." In re Anderson, 471 F.2d 1237, 176 USPQ 331, at 333 (CCPA 1973), emphasis in original. A patentee is not only entitled to narrow claims particularly directed to the preferred embodiment, but also to broad claims which define the invention without a reference to specific instrumentalities. In re Anderson, at 1241 (citing Smith v. Snow, 294 U.S. 1, 11, 24 USPQ 26, 30 (1935)). Further, because "it is manifestly impracticable for an applicant who discloses a generic invention to give an example of every species falling within it, or even to name every such species, it is sufficient if the disclosure teaches those skilled in the art what the invention is and how to practice it." In re Grimme, Keil and Schmitz, 124 USPQ 449, 502 (CCPA 1960). There is, therefore, no requirement for disclosure of every species within a genus. Applicants are entitled to claims that are commensurate in scope not only with what Applicants have specifically exemplified, but commensurate in scope with that which one of skill in the art could obtain by virtue of that which the Applicants have disclosed.

Applicants respectfully submit that the Examiner has not established a *prima facie* case of lack of enablement, as the proper inquiry with respect to scope of enablement under 35 U.S.C. §112, first paragraph, is whether it would require ***undue*** experimentation to make and use the subject matter as claimed. **A considerable amount of experimentation is permissible**, particularly if it is **routine experimentation**. As appreciated by the Examiner, the amount of experimentation that is permissible depends upon a number of factors, which include: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, and the breadth of the claims. Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986); see also In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988).

Analysis

Claim scope and amendments. In response to the Examiner's comments, Applicants have herein amended claim 1 to recite:

"A method for detecting the presence of an a cancer in a particular organ characterized by an increased amount of organ-specific free floating DNA, comprising:

obtaining a bodily fluid sample from a test human having a cancer in a particular organ characterized by an increased amount of corresponding organ-specific free floating DNA relative to that of a normal control value;

measuring an amount or presence of free floating DNA that originates from the[[a]] particular organ in the sample comprising analysing for a DNA methylation pattern that is characteristic for the particular organ; and

comparing the measured amount or presence of free floating DNA that originates from the particular organ of the test human with that of a normal control value, and determining the

presence of a cancer in the particular organ characterized by an increased amount of organ-specific free floating DNA based on an increased measured amount of corresponding organ-specific free floating DNA."

Conforming amendments have also been made to claims 2, 6, 10, 11, and 13.

The claim amendments serve to clarify that the determined cancer is limited to a cancer in a particular organ characterized by an increased amount of corresponding organ-specific free floating DNA relative to that of a normal control value, and that the organ-specific methylation pattern that is measured corresponds to that of the particular cancerous organ, and provide, as suggested by the Examiner, a proper association step to compare results with that of control/normal individuals.

Support for the amendments is found in the originally filed specification. No new matter has been added.

Teachings of specification and state of the art. Regarding the Examiner's above-summarized comments with respect to specification teachings and the state of the art, and regarding any alleged requirement for validation, Applicants contend that the specification in fact teaches that the detection of an organ-specific methylation pattern in the free-floating DNA is indicative a disease of said organ, and that while this result *invites* the practitioner to now adventitiously *focus* on said organ (*e.g.*, when further analyzing the patient's potential disease, or treatments, etc.), whether or not the practitioner employs another *adjunct* method to further confirm or further validate a final diagnosis is discretionary, and there is no teaching in the specification, including the text cited by the Examiner about a *necessity* to perform further analyses to practice the invention as currently claimed. Specifically, the specification states that "[t]he next step *could* be to employ …" (emphasis added). Therefore, it is inappropriate to construe the specification as teaching that validation studies are sometimes needed to associate detection of free floating DNA with detection disease. Obviously, there are options to further

characterize the disease (e.g., with respect to grade, or specific sub-types of disease), as in any other diagnostic method. However, as taught by Applicants, a correlation can be made, for example, between a substantial amount of free floating DNA originating from liver, and the fact that the patient bears a diseased liver, without such further characterization options.

Applicants have previously amended the claims to include a comparison with normal control values.

With respect to the Examiner's comments on Cottrell, Applicants agree that methylation-based studies must have adequate requirements for consistency and performance, and defined clinical questions, sample sets, and methodologies coupled with current methylation technology. Indeed, Applicants maintain their contention that the teachings of the instant specification in combination with the skill in the art provide these benchmark requirements. Cottrell merely emphasizes the importance of precisely the approaches disclosed by the present Applicants. Applicants respectfully point out that the unpredictability associated with technical/methodological issues for detection of methylation differences were sufficiently overcome as of time of filing of the present application. The present Applicants are recognized in the art for highly industrial, sophisticated array-based processes that allow for the simultaneous analysis of thousands of CpG sequences or multiple indications in an efficient, high-throughput manner. Moreover, Applicants have amended the claims as described above to limit the claims to detection of cancer of particular organs and that are accompanied by an increased level of the particular organ-specific DNA in the blood or body fluid.

The source of the whole amount of free floating (i.e. circulating DNA) in blood may be caused by a variety of reasons, such as treatment of the cancer patient with a rather toxic agent (e.g., a chemotherapeutic agent). For example, evidence is discussed in Jahr et al. (*Cancer Res* 61 (2001):1659-1655) (hereinafter "Jahr") that circulating DNA might originate from apoptotic

and necrotic cells, whereas Anker et al. (*Cancer Metastasis Rev.* 18 (1999): 65-73) (hereinafter "Anker") discusses that the origin likely involves "active release," rather than lysis of circulating cancer cells from necrosis or apotosis. In any event, it is irrelevant to the method as claimed whether the entire amount of circulating DNA correlates to cancer or not, because it is the amount of *organ-specific* circulating DNA, which is the analyte of interest, and which is correlated to the presence of a diseased organ.

With respect to the Examiner's statement that the specification only indicates that an increased level of organ specific free floating DNA is indicative of an organ based disease, but not a specific disease, the correlation between circulating DNA level and cancer has been discussed in detail in the specification at pages 8 and 9, where the specification teaches that "elevated levels of circulating DNA appear to be characteristic for most but not all of the carcinoma diseases." Additionally, Applicants' Figure 3 shows results of determining increased free floating DNA levels in serum of patients with various types of cancer relative to normal controls.

The predictability or unpredictability of the art and degree of experimentation. Regarding the Examiner's statements with respect to predictability or unpredictability of the art (in view of Bremnes, Jung, Sidransky, etc.) and degree of experimentation, as discussed herein above, while there may be unpredictable variation in the total amounts of free-floating DNA, such variation is irrelevant for the method as claimed, as Applicants' claimed inventive methods comprise a correlation between organ-specific circulating DNA and disease of said organ. That is, while the clinical value of a total amount of circulating DNA may be questionable or unpredictable, the analysis of organ specific fractions therein is highly informative as disclosed and claimed by the present Applicants.

With respect to the Examiner's comments regarding the teaching of Yates that

methylation can be detected in normal tissue, Applicants respectfully point out that it is well appreciated in the art that methylation patterns may not only be indicative of cancer (neoplasm), but also bear additional information (e.g., aging, development, etc). Applicants' claimed methods, however, use the methylation status of CpG dinucleotide sequences as diagnostic tools where they are methylated in a pattern specific to a particular organ (e.g., regardless of the age status of said organ, see, e.g., page 35, second paragraph.

> "[0133] If a CpG positions is only ever specifically methylated when the corresponding DNA sequence was isolated from one cell type, for example, kidney cells but said CpG position is not methylated when the DNA was isolated from another cell type, for example, liver cells, blood cells, bladder cells or colon cells etc. said CpG position is an 'informative CpG position.' A DNA sequence carrying one or more informative CpG positions in this context is called a 'marker gene', regardless whether it is a gene in the common sense or not."
>
> also in [0170]:
>
> "Those genes contain informative CpG positions, CpG positions that are differentially methylated, specifically for the tissue the DNA has been isolated from."

Yates et al. (2006) (hereinafter "Yates"), and the references therein, refer to the phenomenon of increased methylation of CpG dinucleotides during the process of aging. The investigators compared two groups consisting of cancer-free individuals either under the age of 40 or over the age of 70, respectively, and found that DNA from the second group showed generally higher methylation of a panel of genes. According to the statistics presented by the National Cancer institute (NCI), the median age at diagnosis for cancer of the colon and rectum was 71 years during the years between 2001-2205 (see NCI webpage). Approximately 0.1% was diagnosed under age 20; 1.0% between 20 and 34; 3.7% between 35 and 44; 11.6% between 45 and 54; 18.3% between 55 and 64; 25.1% between 65 and 74; 28.2% between 75 and 84; and 12.2% 85+ years of age. As can be seen from the statistics, and as is generally recognized in the art, the incidence of colon cancer increases with age.

Applicants respectfully point out, however, that this age aspect, as well as other aspects such as recent transplant or trauma (as discussed in Lui, cited by the Examiner), would be reflected in the proper that normal controls as presently claimed.

Amount of direction or guidance provided by the specification. Regarding the Examiner's statements with respect to the amount of direction or guidance provided by the specification, Applicants point out that it is misleading for the Examiner to judge sufficiency of guidance by stating that "the specification does not provide any guidance as how to correlate detection of disease by the detection of free floating DNA," in view of the fact that the specification in fact teaches that an *increased* level of organ-specific circulating DNA is indicative of said diseased organ.

As discussed above , the specification also does not indicate that a correlation *must* include a *validation study* to confirm detection of disease. Rather, the specification teaches that it is an option for the practitioner to further analyze the organ identified as the source of DNA, or alternatively use the DNA sample for further analysis, as for example, by applying a cancer stage-specific markers.

> "[0168] ... Wherein the extracellular DNA can clearly be correlated to a specific organ or tissue as the predominant source a further analysis of said organ or tissue--or a further analysis of said DNA by means of cancer marker genes--as described elsewhere--is highly indicated."

The correlation between circulating DNA level and cancer has been discussed in detail in the specification at pages 8 and 9, where the specification teaches that "elevated levels of circulating DNA appear to be characteristic for most but not all of the carcinoma diseases." Additionally, Applicants' exemplary Figure 3 shows results of determining increased free floating DNA levels in serum of patients with various types of cancer relative to normal controls.

Exemplary Figure 4 shows correlation of methylation patterns to different organs (e.g., Adipose, Breast, Liver, Lung, Muscle, and Prostate).

Exemplary Figure 5 shows how CpG positions in ten (10) different genes can be

identified, that can be used to distinguish between kidney and prostate tissue.

Exemplary Figure 6 shows how specific DNA can be quantified.

Exemplary Figure 7 shows how specific CpG methylation patterns can be used to distinguish four tissues (brain, breast, liver, and muscle).

Examples 1 through 4 are prophetic examples, based on Applicants' specification teachings.

In summary, Applicants point out that with respect to enablement, ALL of the Wands factors must be considered by the Examiner and not merely the *predictability* factor. Additionally, under U.S. Patent Law, a considerable amount of experimentation is permissible, particularly if it is routine experimentation. As appreciated by the Examiner, the amount of experimentation that is permissible depends upon a number of factors, which include: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, and the breadth of the claims. Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986); see also In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988).

Applicants submit, with respect to determination of cancer, that given the knowledge in the art, the teachings of the specification, the steps of obtaining a bodily fluid sample from a test human; determining an amount or presence of free floating DNA that originates from a particular organ in the sample comprising analysing for a DNA methylation pattern that is characteristic for the particular organ; and determining the presence of a disease characterized by an increased amount of organ-specific free floating DNA based on comparing the amount or presence of free floating DNA that originates from the particular organ of the test human, with that of a normal control value, does not amount to undue experimentation. If any experimentation is required to

practice the present claims, such experimentation is merely routine and not undue upon consideration of all of the *Wands* factors.

The Examiner has offered insufficient evidence to support that any such required experimentation is other than routine. As appreciated by the Examiner, the level of skill in the art at the time of filing was and is high, and given the instant teachings and those of the art, determination of the methylation state of one or more CpG residues in free floating DNA, relative to a control, could be done by one of ordinary skill in the art at the time of filing in a matter of a few days or a week using routine, standard DNA manipulation methods and methylation assays available at the time of filing of the present application.

Applicants point out that the current amendments serve to clarify that the determined cancer is limited to a cancer in a particular organ characterized by an increased amount of corresponding organ-specific free floating DNA relative to that of a normal control value, and that the organ-specific methylation pattern that is measured corresponds to that of the particular cancerous organ"—as supported by the specification using organ-specific methylation patterns, with normal/control comparison. In light of the scope of the claims, the teachings in the specification, the presence of specific examples in the specification, the high level of skill of those in this art, the knowledge of those of skill in this art (as exemplified by the Examiner's own cited literature), and the predictability of the subject matter, Applicants respectfully submit that one of skill in the art could readily make and use the presently claimed subject matter without undue experimentation.

Additionally, Applicants contend that in any event, claim 12, is allowable as being directed to "[a] method for determining the fraction of total free floating DNA in a bodily fluid that originates from a specific organ …", and does not recite determination of cancer.

Finally, detection of cancer with methylation patterns in free floating DNA is *not*

unpredictable, as was discussed in Applicants' last Responses and Amendments, where the method has been confirmed by the use of the colon cancer marker Septin 9, for methylation, which has repeatedly and predictably been correlated to colon cancer, and which is currently developed to become an approved blood based colon cancer marker.

This conclusion is not defeated by the Examiner's assertion of Raykan because, for the alleged 80% of CpGs studies by Raykan, there is no reason by a 20% or even greater variation at a given CpG would necessarily destroy the utility of the presently claimed assays because (i) such variable CpGs would be reflected in the "normal control values" recited in the presently amended claims, and (ii) differences in 20% or perhaps greater would be overshadowed by increased levels of free floating DNA, with can be hundreds of fold, as documented in the literature. Additionally, Raykan thus teaches that, even in their sample, 20% of the CpG has no or less than 20% variation, thus in fact further validating the utility of applicants claimed invention.

Additionally, Applicants respectfully take issue with the Examiner's contention that Applicants' claimed invention is unclear because a liver metastasis of a colon cancer (e.g., Paredes-Zaglul) could be indicative of a liver based disease or rather a colon based disease. Applicants submit that if the methylation pattern of the metastasized cells retained the colon pattern, then Applicants assays would still detect a colon based cancer, as claimed, which still would be present in the individual—along with a liver metastasis—and further point out that such an involvement could be detected using liver specific markers, but that this does not defeat the validity of Applicants' claimed assay. It merely points out that, as would be appreciated in the art, it might be prudent to test samples not only with respect to the primary tumor, but also include assays for likely secondary, metastatic cancer organs, where such is indicated based on knowledge in the art. Paredes-Zaglul does not, therefore, render Applicants' claimed invention

unclear, or unenabled, it in fact, further accentuates the need for Applicants' invention, and applications involving more than one organ-specific marker.

Accordingly, for all of the aforementioned reasons, Applicants respectfully submit that the basis for this rejection has been overcome, and request that the rejection be withdrawn.

Rejections under 35 U.S.C. § 103

The Examiner rejected claims 12 and 14, under 35 U.S.C. § 103(c), as allegedly being unpatentable over Dennis in view of Heiskanen.

Specifically, the Examiner (citing Dennis at page 2, paragraph 9; and page 5, paragraph 43) urges that Dennis teaches Applicants' claimed method.

Applicants traverse this rejection, based on the fact that neither Dennis nor Heiskanen teach use of organ-specific methylation patterns as presently claimed.

Dennis does **not** teach the use of organ specific methylation patterns as presently claimed.

Applicants respectfully point out that the present invention is based on the use of organ markers or tissue markers, which are nucleic acids bearing organ or tissue specific methylation patterns, independent from the question of which donor the tissue came from. Whereas, Dennis merely teaches the use of markers that are donor or recipient specific. Specifically, Dennis teaches the use of the androgen receptor gene methylation pattern, which is different between males and females. In this way, the levels of donor DNA in a recipient of the tissue can be followed, this does NOT anticipate Applicants' invention using organ tissue specific DNA markers. Dennis' markers are not organ specific, they are donor specific, and therefore Dennis et al do not anticipate Applicants' claims, which recite use of organ-specific DNA methylation

pattern, and not use of a methylation pattern that would not distinguish between a given donor's own organs.

Nowhere does Dennis teach or suggest the use of organ-specific DNA methylation patterns. Moreover, this deficiency is not cured by combining the teachings of Dennis with those of Heiskanen (teaches a method of taking a target DNA and binding it to a surface (microarray) before using the target to detect expression levels (abstract)).

Applicants, therefore, respectfully request withdrawal of this rejection.

CONCLUSION

In view of the foregoing amendments and remarks, Applicants respectfully request entry of the present Amendment and allowance of the amended claim set provided herein. The Examiner is encouraged to phone Applicants' attorney, Barry L. Davison, to resolve any outstanding issues and expedite allowance of this application.

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